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A Study of the Effectiveness of Hyaluronidase - Alone and in Combination with Other Drugs - In the Prevention or Minimizing of Abdominal Adhesions.

--Alvin W. Mooney, B.A., M.D.

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SYNOPSIS

A relationship between the surgical condition of bowel obstruction and adhesions is presented. This is followed by a brief review of the history of methods tried in the prevention of adhesion formation. Something of the rationale of adhesion formation is dealt with to indicate the basis for investigation of corticoid substances and hyaluronidase as adhesion preventatives.

The main features of hyaluronic acid and the enzyme hyaluronidase are then described. The biochemistry of hyaluronidase is next given.

In the experimental section the investigation is divided into four chapters. The first describes the work of arriving at an optimal dose of talc to cause a standard amount of foreign body adhesions. Hyaluronidase in varying amounts was then used to determine the optimum dosage for adhesion prevention. These results are included.

In the second chapter the standardization of a technique to produce surgical adhesions is presented. The technique of choice was found to be multiple caecal crushes which resulted in temporary vascular embarrassment to a segment of the caecum.

Chapter three presents the effect of hyaluronidase on surgical adhesion formation.

The final chapter on experimental investigation indicates the results of combining the optimum dose of hyaluronidase in three series of rats with an antibiotic, with cortisone and with ACTH.

The final chapters include a discussion of the results obtained and the conclusions drawn therefrom. It is observed that hyaluronidase in a single optimal dose may reduce the incidence of adhesion formation. There still remains a high incidence of adhesion occurrence.

The use of an antibiotic is indicated to be advantageous, whereas the use of corticoid-like substance and adrenal cortex stimulant are observed to result in an adverse effect on adhesion prevention.

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"A Study of the Effectiveness of Hyaluronidase - Alone and in Combination With Other Drugs - in the Prevention or Minimizing of Abdominal Adhesions".

A review of adhesion study and a presentation of some original investigations of hyaluronidase as a preventative for abdominal adhesions post-operative.

by

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INTRODUCTION

The prime objectives of surgery are to postpone death and to increase the comfort of living. One major cause of death is intestinal tract obstruction.

"Gibson[3], in 1900, reviewed one thousand operations for acute intestinal obstruction.....the over-all mortality rate was forty-seven per cent whereas the mortality rate for patients over fifty years of age was fifty-six per cent.... At Grace Hospital between the years of 1945-1950, the over-all mortality rate for the four hundred and twenty-five patients treated was 15.7 per cent. Almost one-fourth of all patients were over the age of sixty. In this age group the mortality rate was found to be twenty-four per cent.....The etiological factors responsible for acute small bowel obstruction has shown a decided change in this fifty-year period. In material presented by Gibson during the ten-year period of 1888-1898, the following etiological factors were reported: Bands - 18.6 per cent. The etiological factor responsible for the acute small bowel obstruction in the series of four hundred and twenty-five treated at Grace Hospital fifty years later is as follows: Adhesions - 56 per cent....".A reduction in the number of patients with intestinal adhesions would do much to further reduce the number of such patients obstructed. This would in turn reduce the mortality rate for acute small bowel obstruction."

In support of the findings of Cantor[6] on the importance of postoperative adhesions as a cause of bowel obstruction are the findings referred to by Parker[20] in his report on Acute Intestinal Obstruction. "More recent studies, however, indicate that adhesions and adhesive bands are the predominant causes of intestinal obstruction today. In a recent series, published by Mersheimer and Winfield, adhesions and bands were the principal causes of obstruction; accounting for 34 per cent of their cases. In Scandinavia, appendectomy was recorded as the most frequent predisposing cause of obstruction, and drainage of the abdominal cavity increased the incidence of ileus ten fold. Dennis states that adhesions and bands are approximately equal in occurrence and more than half the obstructions, due to bands are sequelae of gynecologic surgery. In a recent report by Becker, 90 per cent of a series of 412 cases of bowel obstruction due to adhesions had previously been subjected to laparotomy. Pelvic operations accounted for 34.3 per cent and appendectomy for 28.3 per cent of the cases."

Sawyer[22] states that "obstruction caused by bands and adhesions has been classified into three groups, (1) early post operative (2) late post operative and (3) unoperated cases.....The incidence of early post operative obstruction is much higher than the surgical statistics indicate because the obstructing band or adhesion is usually fresh and friable so that strangulation of the bowel is rare early after an operation."

From the foregoing can be realized the importance of reducing the formation of adhesions. Years ago, Hertzler[4] referred to the hopeless situation regarding adhesions.... "This sequence lasts as long as the patient does, or the hopeful persistence of the surgeon endures". This surgical enigma constitutes a constant professional challenge and a justification for periodic consideration of the subject. Naegeli[4], examined by pneumoperitoneum, 148 cases with previous laparotomies and found that 80 - 90 per cent of them had x-ray evidence of intra-abdominal adhesions. There is one factor in the problem which has received little consideration; namely, the apparent tendency of some individuals to develop adhesions as compared to others.

Boys[4], in 1942, published a review of the history of surgical treatment of obstruction and the role of adhesions and bands as causes of bowel obstruction. He also reviewed the methods that had been employed in trying to reduce adhesion formation. One group of methods included "vaccination" of the peritoneum, bacteriophage therapy and chemotherapy. Chemotherapy included the use of fibrolysin and iodides. Another group of techniques involved the use of various methods to keep surfaces apart. Foils of silver and other metals, silk and rubber sheets, fresh bladder strips, aristol and Cargile membrane were tried as well as amniotic membrane. Allantoic membrane, particularly beef, was reported to be of some value.

Lubricants were given a trial and included mineral oil, olive oil, camphorated oil, fat, agar, gumarabic, gelatin, paraffin, vaseline, collodion and albumin. These only resulted in foreign body reaction within the abdomen.

The use of fluids in the abdominal cavity was tried and included saline of various concentrations, dextrose solution and amniotic fluid. Pneumoperitoneum was also tried.

Digestive substances were used to reduce the fibrin bridgework in advance of fibroblastic activity. These substances included pepsin and trypsin. Papain at one time was widely used.

Again an attempt was made to prevent the coagulation of fibrin. This was attacked through the calcium factor and resulted in the investigation of sodium citrate and sodium oxalate. Peptone was tried as a neutralizer of fibrinogen, hiruden to control thrombokinase activity. None of these were satisfactory.

Boys in association with other workers tried a combination of heparin and saline. This combination he felt to be of value. It never was extensively used because the heparin created the danger of haemorrhage.

Thus by 1942, no adjunct to good surgical technique was available.

Let us now consider some of the surgical techniques important in the reduction of adhesion formation. Trauma has been shown experimentally to be a factor in causing adhesion formation. Gentleness in handling the bowel and other pelvic and abdominal viscera is essential. Foreign bodies such as unnecessary drains, irritating powders and harsh sponges should be avoided. Raw surfaces should be left as small as surgically feasible. Infection should be scrupulously avoided, whenever possible. Blood and bleeding intraperitoneally should be reduced to a minimum before closure of the peritoneum is begun.

The mesothelium, when lost, is reformed. If the underlying connective tissue is damaged, then repair of this tissue is also attempted by the body. This reparative process involves the initial outpouring of fibrin. Fibrin becomes coagulated and in so doing may temporarily fix together in unnatural apposition intra-abdominal surfaces. If mesothelium grows to cover the surfaces and frees them again, then the adhesion is only temporary. However, if at the phase of fibroblastic activity, fibroblasts invade the fibrin bridgework and permanently join the surfaces with a cellular attachment, then permanent adhesion results.

There are three modes of attack on adhesion formation. First, keep the surfaces apart; second, reduce the formation of coagulated fibrin or absorb the fibrin before fibroblastic

activity joins the surfaces together; third, suppress fibroblastic activity or granulation tissue formation until mesothelialization is complete.

We can now proceed to some of the more recent studies on adhesion control. Methods described by Boys to keep the surfaces apart mechanically were unsatisfactory. Schiff[23] and co-workers conducted an interesting experiment based on using the natural motility of the bowel to prevent surfaces remaining long enough in apposition to result in adhesion development. In one group of eight animals treated with Prostigmine methyl sulfate, hypodermically, there was an average of 5.1 adhesions of average density +. Another group of eight animals treated with atrophine hypodermically and kept in a state of starvation, at autopsy showed an average of 17.4 adhesions of average density +++. They concluded that proper pre and post operative care of the bowel was important in minimizing adhesion development.

Certain intra-abdominal structures, such as the omentum and liver, and the organs of the pelvis, cannot be properly stimulated to motility to prevent or minimize adhesion formation. Therefore motility is an incomplete solution to the problem of adhesion formation.

Another approach to the problem of adhesions was developed after the discovery of effects in the body of cortisone and the other adrenal cortical hormones. ACTH stimulates in an individual with adequate adrenal cortex, an increased production of the adrenal corticoid hormones. Cortisone simulates in some measure the action of these naturally produced corticoids. One of the effects of the corticoids is the resolution or suppression of cells of mesodermal origin, such as fibroblasts and those in granulation tissue. It was contemplated therefore, that by medication with ACTH or cortisone, the stage of fibroblastic organization in the fibrin could be suppressed and allow time for mesothelial elements to ensheath the abdominal visceral and parietal structures. The temporary adhesions of fibrin would be prevented from becoming permanent cellular fibrous adhesions.

The action of cortisone however, is limited to the suppression of granulation tissue formation and fibroblastic development, pre-existing tissues are not resolved. Fibrin exudation and coagulation is not prevented.

There are two aspects to the use of cortisone. The first consideration is whether the effect is the one to be anticipated. The other consideration is whether the local effect at the site of potential adhesion development is to be duplicated at the site of incision and retard wound healing, or result in weak wound closure.

Many workers have enthusiastically carried out experimental trials of cortisone treatment on both rats and dogs as well as other animals. These trials have been for the prevention of adhesions after both foreign body irritation and surgical damage. The dose of cortisone has been varied from very small to near toxic amounts. Cortisone has been used pre-operatively and post-operatively, in single doses and serial treatments. It has been used daily continuously, and in interrupted series.

The reports of Hubay[14] and Lyall[15] illustrate the results. There was only a partial suppression of adhesion formation. Pre-operative medication combined with post-operative medication appeared to be more valuable than post-operative medication alone. In some animals a refractory condition developed toward cortisone. In others, adhesion formation was merely delayed and not permanently obviated, particularly in the case of foreign body irritation. Furthermore in many animals they reported inferior wound healing although by microscopy the union was satisfactory. Aldrich[1] reported that based on histological examination and objective tensile tests, the strength of wound union was reduced by cortisone medication because of suppression of the mesenchymal cellular elements.

It might be said then that although cortisone may

have some benefits, the use of this medication is of doubtful value because of the delayed response; the beneficial effect is only minimal; the systemic effect may cause poor wound healing and contribute to reactivation of such conditions as duodenal ulcer and arrested tuberculosis. A further factor in the failure of cortisone to prevent adhesion formation, is probably a result of mesothelium itself being of mesenchymal origin, and susceptible to the suppressive effect of cortisone.

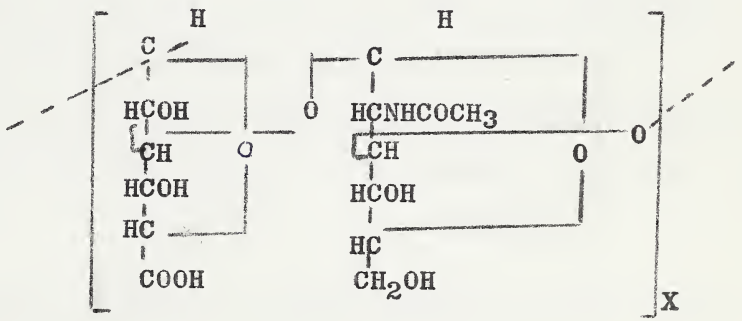
Earlier it was indicated that three modes of combating adhesions present themselves: keeping surfaces apart, preventing temporary adhesions, and finally suppressing fibroblastic invasion of fibrin to form permanent adhesions.

We have noted that no successful means exist for the first mode. We have noted that cortisone, the best prospect to date has failed in the trials at the final mode of control. Another route of attack at the second mode of control has presented itself in recent years. Hyaluronidase was found to have an action on ground substance through its effect on hyaluronic acid. It was hoped that this enzyme would act locally to cause absorption of the fibrin coagulum and thus aid in removal of the material before temporary adhesions developed and became a bridge for the fibroblasts to cross from surface to surface.

Considerable research has been done on the use of hyaluronidase. Some results have been favorable, some unfavorable. The present research has been undertaken to establish to our satisfaction what the status of hyaluronidase is as a preventative of adhesion formation.

Hyaluronic acid[5], a sulfate-free polysaccharide, is found in the vitreous humor, synovial fluid, skin, umbilical cord, and in general (together with protein and other mucopolysaccharides) forms the so-called "ground substances" of the mesenchyme. Hyaluronic acid is known to be composed of alternating units of glucuronic acid and N-acetyl-glucosamine.

A recently proposed structure for the repeating unit in this polymer is shown.



In chondroitin sulfuric acid the terminal H of the terminal C of glucosamine is replaced by SO_3H to become CH_2OSO_3H. It will be noted that in both the inter-saccharide linkages are B-1,3. As in the case of most mucopolysacchides, the exact molecular weight of hyaluronic acid in its native state is not known.

The mucopolysaccharides of the various mucous membranes throughout the body, as integral parts of mucoproteins, are thought to have a protective influence on the tissues which secrete the mucopolysaccharides.

Hyaluronidase is classified as a polysaccharidase, type hetero polysaccharidase. It is also described as a mucopolysaccharidase or mucase. Cantarow[5] believes that it is probable at the present time that the commonly used enzyme preparations are quite heterogenous. The general course of hydrolysis of hyaluronic acid is thought to pass through a series of stages which are analagous to those occurring during the hydrolysis of starch by amylase, that is a series of dextrins - in this case mucodextrins - followed by a stage of muco-oligo-saccharides, leading finally to glucuronic acid and acetylglucosamine. There is some evidence that these intermediate stages involve the action of separate enzymes. The exact role of hyaluronidase will probably soon be established.

Further he suggests certain mucolytic enzymes found in animal tissues catalyse the breakdown of the large polysaccharid molocules. In the tissues, this phenomenon results in decreased viscosity and increased permeability, and these enzymes are sometimes designated "spreading factors". Hyaluronidase falls into this category.

Preferred reading on hyaluronidase is supplied in the report by McClean[16]. Other reports on investigations of hyaluronidase and spreading factors are available from Meyer[18], Chain[7] and Tanturi[24], however the work of McClean is more searching, better rationalized and presented in a more lucid manner.

"Aqueous extracts of mammalian testicle contain a factor that dramatically increases the permeability of the tissues to injected fluids (McClean, 1930,1931; Hoffman and Duran-Reynals, 1931) This can be demonstrated by the rapid disappearance of the BLEB following intracutaneous injection of these extracts and by the rapid spread through a large area of skin of any suitable indicator that may be injected together with them. This factor is associated with the germinal epithelium and can be extracted from spermatozea. (McClean, 1931). Factors with similar diffusing properties have been obtained from the most diverse sources, for example, from filtrates and extracts of staphylococcus and steptococcus (Duran-Reynals, 1933), from organisms of the gas-gangrene group and virulent pneumococci (McClean, 1936), from extracts of malignant tissues (Duran-Reynals and Stewart, 1931; Boyland and McClean, 1935), from snake and spider venoms (Duran-Reynals, 1939) and from leeches (Claude, 1937)."

The first quality of the enzymatic spreading factor which was investigated was optimum pH. Spreading factor or hyaluronidase derived from testicular extract and that derived from Cl. welchii, Vibrion septique and Russell's viper venom, is most active in a medium of pH 4.6. The enzyme from all sources rapidly lost activity at a more acid pH. On the alkaline side of pH 6.5 hyaluronidase from viper venom became completely inactive. The hyaluronidase from other sources showed a reduction in activity as the pH changed from 4.6 toward neutrality but still retained some activity at pH 8.5.

As so often is the case of enzymes of significance to human metabolism, the optimum pH is apparently well away from that at which the enzyme is likely to be functioning. This perhaps is fortunate, thus permitting the body to exercise greater control over the reaction of the enzyme.

It was found that when the concentration of the enzyme from different sources was adjusted so that the reaction time for the reduction of viscosity was comparable, the amount of enzyme necessary to liberate measurable quantities of reducing substances was approximately the same, whatever the source of the enzyme. However, in another series, McClean found that testicular enzyme liberated four times

as much reducing substances from the substrate as did enzyme from other sources under similar circumstances. Then again he found the enzyme from Cl welchii to be twice as effective as testicular enzymes.

Again illustrating variations in the enzyme according to source was the inability of McClean to establish an antigenic effect from purified testicular hyaluronidase. Antisera however, have been established for the enzyme from each of the other sources. These are very specific and control reduction in spread as well as viscosity variation. However, an animal has a limited ability to inhibit injections of the enzyme.

As a result of investigation of electrolyte influence on enzyme activity, the chloride ion was established as the accelerating ion. The sodium ion exerts a depressing effect on the activity of the enzyme. The influence of each varies according to the concentration of the ion.

No relation was established between the time for reduction of viscosity of solutions of hyaluronic acid and the amount of substrate altered at that concentration. Thus at a commencing concentration of 6.6 mg. hyaluronic acid per ml., to reach half viscosity only 20 to 24 per cent of substrate is altered. However at one-tenth of this concentration, in order for the viscosity to be reduced to half, nearly 50 per cent of the substrate is altered.

Further experimental investigation revealed considerable variation, according to source, of hyaluronidase activity on the polysaccharides other than hyaluronic acid. This activity was not significant or consistent. Hyaluronidase activity is not the result of amylase or phosphatase activity.

Certain non-enzymatic substances cause slow spreading effect in the skin. Their action is much more delayed than that of hyaluronidase and is never as extensive. Furthermore the non-enzymatic combination, ascorbic acid and hydrogen peroxide, was found to have a viscosity reducing effect on solutions of hyaluronic acid. This activity did not result in the production of acetylhexosamine as with hyaluronidase. Hyaluronidase added to the preparation after the viscosity reduction effect had been obtained caused the formation of reducing substances so that the presence of ascorbic acid was not a deterrent.

McClean further found that the addition of hyaluronidase to cultures of Cl welchii accelerated the growth of the organism and greatly increased the amount of hyaluronidase produced during growth by the organism.

From the foregoing, certain factors are noted. Hyaluronidase may vary according to source. It may cause a series of reactions to occur, or hyaluronidase may be a

combination of enzymes. The extent of the reaction may vary according to the pH of the substrate. Electrolyte concentration and combination may vary the results obtained.

There are three criteria which hyaluronidase fulfills: It causes rapid increased spreading, it decreases the viscosity of hyaluronic acid solutions, its continued reaction results in the formation of reducing substances.

Antisera to hyaluronidase are known. These limit the action of the enzyme. Cole[9] and associates in an interesting piece of work demonstrated a rise of anti-hyaluronidase in the blood of rabbits post-operatively. This rise commenced immediately post-operatively and reached its maximum on the third to fifth day. By the seventh or eighth day the level had receded back to normal pre-operative level. The antihyaluronidase tended to reduce or inhibit the spreading effect of subcutaneously injected hyaluronidase.

Chandy[8] in 1946, reported the results of using hyaluronidase intraperitoneally to control adhesion formation. He reported his results as significant and sixty per cent successful. Later in 1950, Thomas[25], who worked further on the influence of hyaluronidase intraperitoneally, reported - "No statistically significant

effects were obtained to support the positive results previously reported by two of us".

In 1951, Connelly[10] reported on his investigation of the use of hyaluronidase intraperitoneally after surgical lysis of intraperitoneal adhesions. As a result of his work on dogs he stated - "It has been observed that intestinal adhesions did not form in the presence of hyaluronidase" - and further - "When used in sufficient amount in the peritoneal cavity, hyaluronidase solution rather remarkably prevented the reformation of the lysed adhesions in all of the dogs studied."

As a follow-up in 1953, Wilder[26] reported on his investigation of the results of intraperitoneal hyaluronidase used in rats after surgical lysis of adhesions.(quote) "There was no difference in the reformation of adhesions in the rats treated with hyaluronidase and in those treated with saline".

The use of hyaluronidase experimentally has continued but the work has been done largely in continental Europe and has not been reported in journals available through local channels. It was considered that because of the indecisive status of hyaluronidase findings, further investigation should be carried out locally. If hyaluronidase is effective, then its benefits should be available. If it is not of value, then such should be decided and further consideration ended.

CHAPTER I

The first phase of the undertaking was to establish the most satisfactory method of causing adhesions. It was felt that the procedure decided upon should result in almost one hundred per cent frequency of occurrence, yet in so doing, cause not too much metabolic disturbance. The intraperitoneal injection of turpentine was considered as also was the injection of formaldehyde. However, the disturbance was felt to be too severe for our purposes.

It was finally decided that as in other investigations we should use talc as our irritant. Various weights of talc were considered. It was thought to be necessary to establish at what time the adhesions had become established and fibrosed.

Consequently ten samples each of talc 62.5 mg., 125 mg., 250 mg., and 500 mg., were weighed out. These forty samples were in clean test tubes, closed with cotton wads. The tubes were then autoclaved. Care had to be exercised to keep the tubes and powder dry to avoid caking.

Albino Wistar rats were selected in the weight range of 175 to 250 grams. Females were used exclusively. This was decided because in cases of obstruction the percentage of female patients has been rapidly increasing. Pelvic

laparotomy is a common operation for upper genital tract disease. Adhesions and obstruction are a frequent result.

In the first group of talc samples an attempt was made to form a suspension in distilled water for injection purposes. The larger samples, 250 mg. and 500 mg., did not disperse well in the water and injection was found to be difficult and wasteful. Further consideration caused us to feel that open laparotomy would be more accurate and definite. This also had the advantage of resembling more closely surgical conditions. There would be uniformity of procedure for the foreign body type of adhesions and those to be created surgically later.

The final decision in the first phase for causing a typical intra-abdominal adhesion was to use in four series of nine rats, amounts of 62.5, 125, 250 and 500 mgs. of talc respectively. The talc was delivered intraperitoneally through a funnel placed in a midline incision. Dispersal of the talc was gained by placing 5 cc. of distilled water in the abdomen just prior to completing closure of the peritoneum. The animals were then sacrificed at varying times of 2,4,6,8,11,13,20,27 and 35 days. One of each series was sacrificed at each time. Each rat was autopsied. Results are tabulated in Table I.

TABLE I

Day of Sacrifice	Number and Density of Adhesions with talc.			
	62.5mg.	125mg.	250mg.	500mg.
2nd day	+	+	0	2+
4th day	+	+	3+	4+
6th day	0	+	3+	3+
8th day	+	2+	4+	6+
11th day	+	+	3+	5+
13th day	+	+	4+	5+
20th day	2+	2+	2+	4+
27th day	0	+	2+	5+
35th day	2+		2+	4+

It was decided to abandon a program of weighting the extent and density of the adhesions. This was not considered to be of major significance. The presence or absence of adhesions is the important consideration. Altering the count according to whether the adhesions are 3+ or 1+ brings in a large subjective element.

As a result of our observations it was decided that talc 1/4 gram or 250 mg., was the amount most suitable and that ten days approximately was a reliable duration to wait post-operatively to evaluate adhesion formation. With this combination, adhesion formation was almost one hundred per cent, frequently multiple and adequately mature.

Now that a standard procedure had been developed for causing adhesion formation, the next step was to find the optimum dosage and routine of administration of hyaluronidase to influence the incidence of adhesions.

It was decided to use hyaluronidase in the amounts of 25 units, 50 units, 75 units, 100 units, and 150 units, intraperitoneally. Fifty rats were subjected to laparotomy and intra-abdominally 250 mg. of sterilized talc powder was added to each. Then in the first ten, 25 units of hyaluronidase in 5 cc. of distilled water was added; in group B of ten rats, 50 units was used and so on, such that in group E, 150 units in 5 cc. distilled water was added to each rat.

Each group was further subdivided into two lots of five rats each. In the one lot only a single treatment was used. In the other lot of five, daily injections intraperitoneally of the same amount and concentration of hyaluronidase was used as in the original dose at laparotomy.

All remaining living rats were sacrificed on the day following the tenth injection of the hyaluronidase to the five sub-groups of five each. This was the eleventh post-operative day.

All rats, except one, were autopsied and the adhesions found are recorded on the following tables. In this group one rat was found to be pregnant.

TABLE IIA

SPECIMEN	Talc 250 mg. + 1 treatment 25u Hyaluronidase with 5 cc. D.W.						
	LL	O.toL.	O.toB.	O.toW.	P.O.toB	P.O.toW.	Other
A	-	-	x	-	x	-	x
B	-	x	-	-	-	x	-
C	-	-	x	-	-	-	x
D	-	x	-	-	-	-	-
E	-	x	-	-	-	x	-

5 rats - 10 adhesions Average 2.

Abbreviations: LL - Liverlobes
O.toL. - Omentum to Liver
O.toB. - Omentum to Bowel
O.toW. - Omentum to Wall
P.O.toB. - Pelvic organs to Bowel
P.O.toW. - Pelvic organs to Wall
x - Adhesion

SPECIMEN	Talc 250 mg. + 10 treatments 25u Hyaluronidase with 5 cc. D.W.						
	LL	O.toL.	O.toB.	O.toW.	P.O.toB	P.O.toW.	Other
F	x	x	x	-	-	-	-
G	-	-	-	-	-	-	-
H	-	x	x	-	-	-	-
I	-	-	-	-	-	x	-
J	-	x	-	-	-	x	-

5 rats - 8 adhesions Average 1.6

IIB

SPECIMEN	Talc 250 mg. + 1 Treatment 50 u. Hyaluronidase with 5 cc. Distilled Water.						
	LL	O.toL	O.toB.	O.toW	P.O.toB	P.O.toW.	Other
K	-	x	-	-	-	-	-
L	-	-	-	-	-	x	-
M	-	-	-	-	-	-	-
N	-	x	-	-	x	-	x
O	-	x	-	-	-	-	-

5 rats 6 adhesions average 1.2
1 rat - no adhesions

SPECIMEN	Talc 250 mg. + 10 Treatments 50 u. Hyaluronidase with 5 cc. D.W.						
	LL	O.toL	O.toB.	O.toW	P.O.toB	P.O.toW	Other
P	-	-	x	-	x	-	-
Q	-	x	x	x	-	-	-
R	-	-	-	-	-	-	-
S	-	x	-	-	-	x	x
T	-	x	-	-	-	x	-

5 rats 10 adhesions average 2
1 rat - no adhesions

II C

SPECIMEN	Talc 250 mg. + 1 Treatment 75 u. Hyaluronidase with 5 cc. D.W.						
	LL	O.toL	O.toB	OtoW	P.O.toB	P.O.toW	Other
U	-	x	-	-	-	-	-
V	-	x	-	-	-	-	-
W	-	-	-	-	-	-	-
X	-	x	-	x	-	-	x
Y	-	x	x	-	-	-	-

5 rats 7 adhesions average 1.4
1 rat - no adhesions

SPECIMEN	Talc 250 mg. + 10 treatments 75 u. Hyaluronidase with 5 cc. D.W.						
	LL	O.toL	O.toB	O.toW	P.O.toB	P.O.toW	Other
AA	-	x	-	-	-	-	-
BB	-	x	-	-	x	-	-
CC	-	x	-	-	x	-	-
DD	-	x	-	-	-	-	-
EE	-	x	-	-	x	-	-

5 rats 8 adhesions average 1.6

II D

SPECIMEN	Talc 250 mg. + 1 treatment 100 u. Hyaluronidase with 5 cc. D.W.						
	LL	O.toL	O.to B	O.toW.	P.O.toB	P.O.toW	Other
FF	-	x	-	-	x	-	x
GG	-	-	-	-	-	-	x
HH	-	x	-	-	-	-	x
II	-	-	-	-	x	-	-
JJ	-	-	-	-	-	-	-

5 rats 7 adhesions average 1.4
1 rat - no adhesions

SPECIMEN	Talc 250 mg. + 10 treatments 100 u. Hyaluronidase with 5 cc. D.W.						
	LL	O.toL	O.toB	O.toW.	P.O.toB	P.O.toW	Other
KK	-	x	x	-	-	-	-
LL	-	x	-	-	x	-	-
MM	-	x	-	-	-	x	-
NN	-	x	-	-	-	-	-
OO	-	-	-	-	-	-	-

5 rats 7 adhesions average 1.4
1 rat - no adhesions (escaped)

II E

SPECIMEN	Talc 250 mg. + 1 treatment 150 u. Hyaluronidase + 5 cc. D.W.						
	LL	O.toL	O.toB	O.toW	P.O.toB	P.O.toW.	Other
PP	-	-	-	-	x	x	-
QQ	-	x	x	-	-	-	-
RR	-	x	-	-	x	-	-
SS	-	x	-	-	-	-	-
TT	-	x	x	-	x	x	-

5 rats 11 adhesions average 2.2

SPECIMEN	Talc 250 mg. + 10 treatments 150 u. Hyaluronidase + 5 cc. D.W.						
	LL	O.toL	O.toB	O.toW	P.O.toB	P.O.toW.	Other
UU	-	x	x	-	x	-	x
VV	-	-	-	-	x	-	-
WW	-	-	-	-	x	-	x
XX	-	x	-	-	-	-	x
YY	-	x	-	x	-	-	x

5 rats 12 adhesions average 2.4

II F

Ulceration of Abdominal Wall
at incision

Talc 250 mg. + 5 cc. distilled water + Hyaluronidase									
xltr.	x10tr.	1tr.	10tr.	1tr.	10tr.	1tr.	10tr	1tr.	10tr.
--	--	--	--	--	--	FF	--	--	--
--	--	--	--	--	--	--	LL	QQ	--
--	--	--	--	W	--	HH	--	--	WW
--	I	N	S	--	--	--	--	SS	XX
--	--	--	--	--	EE	--	(escp)	--	YY
0	1	1	1	1	1	2	1	2	3

As a result of these observations it was decided that 100 units of hyaluronidase was most effective although many adhesions were formed.

The next phase was to compare the effect in a series of twenty rats receiving 1/4 gram of talc and 100 units of hyaluronidase, in 5 cc. distilled water at operation and daily intraperitoneal injections of 100 units of hyaluronidase, in 5 cc. distilled water - with a series of twenty rats, which at surgery received intraperitoneally 1/4 gram talc and 5 cc. of distilled water and daily injections intraperitoneally of 5 cc. distilled water.

These rats (40) were sacrificed on the 11th post-operative day and autopsied. The results are shown on Table III.

SPECIMEN	Talc 250 mg	10 treatments	5 cc. D.W.				
	LL	O.toL	C.toB	O.toW	P.O.toB	P.O.toW.	Other
A1	-	-	-	-	-	-	-
B1	-	x	-	-	-	-	-
C1	-	x	-	-	-	-	-
D1	-	-	-	-	x	-	-
E1	-	x	-	-	-	-	-
F1	-	-	-	-	-	-	x
G1	-	-	-	-	-	x	-
H1	-	-	-	-	-	-	-
I1	-	x	-	-	-	-	-
J1	-	-	-	-	-	x	-
K1	-	-	-	-	-	x	-
L1	-	x	-	x	-	-	-
M1	-	x	-	-	-	-	-
N1	-	x	-	-	x	-	-
O1	-	x	-	-	-	-	x
P1	-	x	-	-	x	x	-
Q1	-	x	-	-	x	-	x
R1	-	x	-	-	-	-	-
S1	-	x	-	-	x	-	x
T1	-	-	-	-	-	-	-
	0	12	0	1	5	4	4

20 RATS - 26 ADHESIONS
3 RATS - NO ADHESIONS

AVERAGE 1.3

III B

SPECIMEN	Talc 250 mg. + 10 treatments 100 u. Hyaluronidase with 5 cc. D.W.						
	LL	O.toL	O.toB	O.toW	P.O.toB	P.O.to W	Other
A2	-	x	-	-	-	-	x
B2	-	x	-	-	-	-	x
C2	-	-	x	-	x	-	-
D2	-	-	x	-	x	-	-
E2	x	-	x	-	-	x	-
F2	-	-	-	x	-	-	-
G2	-	x	-	-	-	-	-
H2	-	x	-	-	-	-	x
I2	-	x	-	-	-	x	-
J2	-	x	x	-	x	-	x
K2	-	x	x	-	-	x	-
L2	-	x	-	-	-	-	-
M2	x	x	-	-	-	-	-
N2	-	-	-	-	-	-	-
O2	x	x	-	-	-	-	-
P2	-	-	-	-	-	-	-
Q2	-	x	-	-	-	-	-
R2	-	x	-	-	-	-	-
S2	-	x	-	-	-	-	-
T2	x	-	x	-	-	-	x
U	4	13	6	1	3	3	5

20 rats 35 adhesions average 1.75
2 rats - no adhesions

Another series of twenty rats were treated at surgery with 1/4 gram talc and 100 units of hyaluronidase in 5 cc. of distilled water. There was set up as a control a series of twenty rats which at laparotomy received identical treatment except that none received hyaluronidase. No injections were given post-operatively.

All rats were sacrificed on the eleventh post-operative day and autopsied. The results are given in Table IV.

IV A

SPECIMEN	Talc 250 mg. + 1 treatment with 5 cc. Distilled Water						
	LL	O.toL	O.toB	O.toW	P.O.to B	P.O.toW	Other
A3	-	-	-	-	-	x	-
B3	-	-	-	-	-	x	-
C3	-	-	-	-	-	-	-
D3	-	-	x	x	-	-	-
E3	-	-	-	-	-	-	-
F3	-	-	-	-	-	-	-
G3	-	-	-	-	x	-	x
H3	x	-	-	-	-	x	-
I3	-	-	-	-	x	-	-
J3	-	-	-	-	-	-	x
K3	-	-	-	-	-	-	x
l3	-	-	x	-	-	-	-
M3	-	x	-	-	-	-	x
N3	-	-	-	-	-	-	x
O3	-	-	-	-	-	x	-
P3	-	-	-	-	-	x	-
Q3	-	-	-	-	-	x	-
R3	-	-	-	-	-	x	-
S3	-	-	-	-	-	-	-
T3	-	-	-	-	x	x	-
	1	1	2	1	3	8	5

20 Rats 21 Adhesions Average 1.05
4 rats - no adhesions

IV B

SPECIMEN	Talc 250 mg. + 1 Treatment 100 u. Hyaluronidase with 5 cc. D.W.						
	LL	O.toL	O.toB	O.toW	P.O.toB	P.O.toW	Other
A4	-	-	-	-	-	-	x
B4	-	-	-	-	-	-	x
C4	-	-	-	-	-	-	x
D4	-	-	-	-	-	x	-
E4	-	-	-	-	-	-	-
F4	x	-	-	-	-	-	-
G4	-	-	-	-	-	x	-
H4	-	-	-	-	-	x	-
I4	-	-	-	-	-	x	-
J4	-	-	-	-	-	x	-
K4	-	-	-	-	-	x	-
L4	-	-	-	-	-	x	-
M4	-	-	-	x	-	x	-
N4	-	-	-	-	-	-	-
O4	-	-	-	-	x	-	-
P4	-	-	-	-	-	-	-
Q4	-	-	-	-	-	-	-
R4	-	-	-	-	-	-	-
S4	-	-	x	-	-	-	x
T4	-	-	-	-	-	-	-
	1	0	1	1	1	8	4

20 Rats 16 adhesions Average 0.80
6 Rats - no adhesions

Because Connolly[10] had used large dosage of hyaluronidase a further series of rats was set up.

A series of twenty rats was subjected to laparotomy and the instillation intraperitoneally of 1/4 gram of talc. One group of five received also 2000 units of hyaluronidase in 5 cc. distilled water. The second group of five, received 1000 units of hyaluronidase and the third group of five received 500 units of hyaluronidase. The last group of five received only distilled water with the talc.

All rats were sacrificed on the eleventh post-operative day and autopsied. The results of this series are shown in Table V.

V A

SPECIMEN	LL	O.toL	O.toB	O.toW	P.O.toB	P.O.toW	Other
Talc 250 mg. + 1 Treatment 5 cc. Distilled Water (a) no Hyaluronidase							
A5	-	-	-	-	-	-	-
B5	-	x	-	-	-	-	-
C5	-	-	-	-	-	-	-
D5	-	x	-	-	-	x	-
E5	-	x	-	-	-	-	-
		3	-	-	-	1	-
(b) with 500 u. Hyaluronidase Total 4 Average 0.8							
V B							
F5	-	-	-	-	-	-	-
G5	-	-	-	x	-	-	-
H5	-	x	-	-	-	-	-
I5	-	-	-	-	-	-	-
J5	-	x	-	x	-	-	-
		2	-	2	-	-	-
(c) with 1000 u. Hyaluronidase Total 4 Average 0.8							
V C							
K5	-	-	x	-	-	-	-
L5	-	-	-	-	-	-	-
M5	-	x	x	-	-	-	-
N5	-	x	-	-	-	-	-
O5	-	x	-	-	-	-	-
	-	3	2	-	-	-	-
(d) with 2000 u. Hyaluronidase Total 5 Average 1.0							
V D							
P5	-	x	-	-	-	-	-
Q5	-	-	-	-	-	-	-
R5	-	x	-	-	-	-	-
S5	-	-	-	-	-	-	-
T5	-	-	-	x	-	-	-
	-	2	-	1	-	-	-

Total 3 Average 0.6

Chapter II

The second phase of the investigation was to discover which type of surgical adhesion was most constant in order to evaluate how effective hyaluronidase was against the formation of surgical type adhesions.

Two methods of injuring the bowel were considered. The first was caecal crushing. For this purpose a curved Kelly forcep crush was chosen. The crush was made longitudinally along the mesenteric aspect. The forcep was left closed on the bowel for ten seconds. The alternate method of injury was to strip the peritoneum from the caecum. This was found to be difficult because of the fragility of the bowel and the ease with which injury of the muscular and mucosal layers occurred. The two methods were each used in four rats.

Then too, consideration was given to the method of closure of the abdominal peritoneum. Is inversion or eversion of the wound peritoneum margins preferable, or a factor in adhesion formation? Two of the crush cases were closed by each method. Then two each of the caecal stripping rats were closed by each method.

These rats were all sacrificed on the 10th day and autopsied. The results are shown in Table VI.

VI

SPECIMEN	Treatment	LL	OtoL	OtoB	OtoW	P.O.toB	P.O.toW	Other
A6	Eversion & Crushing of Caecum	-	-	-	-	x	-	x
B6	Same as A6	-	-	-	-	-	-	-
C6	Inversion & Crushing of Caecum	-	-	-	-	x	-	-
D6	Same as C6	-	-	x	-	-	-	-
E6	Eversion & Stripping of Caecum	-	-	-	-	-	-	x
F6	Same as E6	-	-	-	-	x	-	-
G6	Inversion & Stripping of Caecum	-	-	-	x	x	-	-
H6	Same as G6	-	-	-	-	-	-	-

With inversion 1 of 4 with adhesion to incision

With eversion 0 of 4 with adhesion to incision

3 of 4 with Crushing produced 4 adhesions

3 of 4 with Stripping produced 4 adhesions

No conclusion could be reached regarding closure as both inversion and eversion had resulted in smooth peritoneal lining of the peritoneal cavity. Good union was obtained in each method of closure. The fine filamentous adhesion to the incision in one rat closed by inversion, contributed to the decision to continue closure by eversion.

Stripping was discarded as unsatisfactory. The prospect of infection being a factor if sub-visible tears were to occur in the caecal wall. Crushing was selected.

To test the effectiveness of hyaluronidase against surgical types of adhesions a series of thirty rats was set up. All were subjected to laparotomy with a 10 second longitudinal Kelly crush of the caecum along the mesenteric aspect of the caecum. Care was exercised not to occlude the ileo-colic junction. Ten rats were closed with 5 cc. distilled water in the abdomen. The next ten were closed with 5 cc. distilled water and 100 units of hyaluronidase intra-peritoneal. The final ten were left with 5 cc. distilled water and 2000 units of hyaluronidase intra-abdominal.

All rats were sacrificed on the eleventh day and then autopsied. Results are shown in Table VII.

VII A

SPECIMEN	Single Kelly 10 sec. Caecal Mesenteric Border Longitudinal Crush + 5 cc. D.W. with 100 u. Hyaluronidase.						
	LL	O.toL	O.toB	O.toW	P.O.toB.	P.O.toW	Other
A7	-	-	-	-	-	x	-
B7	-	-	-	-	-	-	-
C7	-	-	-	-	-	x	-
D7	-	-	-	-	-	-	-
E7	-	-	-	-	-	-	-
F7	-	-	-	-	-	-	-
G7	-	-	-	-	-	-	-
H7	-	-	-	-	-	-	-
I7	-	-	-	-	-	x	-
J7	-	-	-	-	-	-	-
	0	0	0	0	0	3	0

10 Rats 3 adhesions Average 0.3

7 Rats no adhesions

VII B

SPECIMEN	Single Kelly 10 sec. Caecal Mesenteris border longitudinal Crush + 5 cc. D.W. with 2000 u. Hyaluronidase.						
	LL	O.toL	O.toB	O.toW	P.O.toB	P.O.toW	Other
A8	-	-	-	x	-	-	-
B8	-	-	-	-	-	-	-
C8	-	-	-	-	-	-	-
D8	-	-	-	-	-	-	-
E8	-	-	x	-	x	-	-
F8	-	-	-	-	-	-	-
G8	-	-	x	-	-	-	-
H8	-	-	-	-	-	-	-
I8	-	-	-	x	-	x	-
J8	-	-	-	-	-	-	-
	0	0	2	2	1	1	0

10 Rats 6 Adhesions Average 0.6
6 Rats no - Adhesions

VII C

SPECIMEN	Single Kelly 10 sec. Caecal Mesenteric border longitudinal Crush + 5 cc. Distilled Water						
	LL	O.toL	O.toB	O.toW	P.O.toB	P.O.to W	Other
A9	-	-	-	-	-	-	-
B9	-	-	-	-	-	-	-
C9	-	-	-	-	x	-	-
D9	-	-	-	-	-	-	-
E9	-	-	-	-	-	-	-
F9	-	-	-	-	x	-	-
G9	-	-	-	-	-	-	-
H9	-	-	x	-	x	-	-
I9	-	-	-	-	-	-	x
J9	-	-	-	-	-	-	-
	0	0	1	0	3	0	1

10 Rats 5 Adhesions Average 0.5
6 Rats - no adhesions

A single longitudinal crush of 10 seconds of the mesenteric aspect of the caecum was inadequate. Several of the control rats failed to show adhesions. Therefore another series of rats was set up to find an effective crush method of causing caecal adhesions.

Twelve rats were subjected to laparotomy and in six there was a longitudinal crush of about 1 cm. made along the mesenteric border of the caecum. Care was exercised not to occlude the ileo-colic junction. A large curved Kelly forcep was used. The other six rats were given a cross caecal crush with a similar forcep. All crushes were of 20 seconds duration. At closure 5 cc. distilled water was left in the abdomen of each rat.

The rats were sacrificed on the eleventh day and autopsied. The results are shown in Table VIII.

VIII A

SPECIMEN	Single Kelly 20 sec. Caecal mesenteric border longitudinal Crush + 5 cc. Distilled Water.						
	LL	O.toL	O.toB	O.toW	P.O.toB	P.O.toW.	Other
A10	-	-	-	-	-	-	-
B10	-	-	-	-	-	-	-
C10	-	-	-	-	x	-	-
D10	-	-	-	-	x	-	-
E10	-	-	-	-	x	-	-
F10	-	-	-	x	-	-	-

6 rats 4 adhesions Average 0.67

VIII B

SPECIMEN	Single Kelly 20 sec. Cross Caecal Crush + 5 cc. D.W.						
	LL	O.toL	O.toB	O.toW	P.O.toB	P.O.toW	Other
G10	-	-	x	-	-	-	-
H10	-	-	-	-	-	-	-
I10	-	-	-	-	-	-	-
J10	-	-	-	-	-	-	x
K10	-	-	-	-	-	-	-
L10	-	-	-	-	-	-	-

6 RATS 2 ADHESIONS Average 0.33

The results were unsatisfactory and a further series was deemed necessary. One is surprised at the amount of damage the bowel can sustain without permanent injury and adhesion development.

In the next series, twelve rats were again used. All were subjected to laparotomy. In six animals the crush technique was used. Two crushes were made; one transverse of the bowel just distal to the ileo-colic junction and the other as before, along the mesenteric aspect of the caecum.

This combination of crushes, each for 20 seconds, left a segment of the distal portion of the caecum with temporarily impaired vascular supply.

The other six rats had peritoneal stripping performed on the caecum. At closure 5 cc. of distilled water was left in the abdominal cavity of each rat.

After eleven days the rats of this series were sacrificed and then autopsied. Results are shown in Table IX.

IX A

SPECIMEN	Multiple (2) Kelly Caecal Crush + 5 cc. of Distilled Water						
	LL	O.toL	O.toB	O.toW	P.O.toB	P.O.toW	Other
All	-	-	-	-	x	-	-
Bll	-	-	-	-	x	-	-
Cll	-	-	-	-	x	-	-
Dll	-	-	-	-	x	-	-
Ell	-	-	x	-	x	-	-
Fll	-	-	x	x	x	-	-

6 rats 9 adhesions Average 1.5
(All rats with adhesions 1 P.O.toBowel)

IX B

SPECIMEN	Abrasion of Caecum + 5 cc. Distilled Water.						
Gll	-	-	x	-	-	-	-
Hll	-	-	-	-	x	-	-
Ill	-	-	x	-	x	-	-
Jll	-	-	x	-	-	-	-
Kll	-	-	x	-	x	-	-
L12	-	-	x	x	-	-	-

6 rats 9 adhesions Average 1.5

The multiple twenty-second crush technique resulted uniformally in the formation of caecal adhesions. This method was selected for causing surgical adhesions for testing hyaluronidase.

Chapter III

For the test of effectiveness of hyaluronidase against surgical adhesion formation, a further group of twenty-four rats were subjected to laparotomy. Four were given the standard multiple caecal crush and on closure 5 cc. of distilled water was left intraperitoneal. These four, along with the six in the previous Table, to constitute the ten control rats. Of the remaining twenty rats similarly treated with multiple caecal crush - ten at closure had 5 cc. of distilled water and 100 units of hyaluronidase left intra-abdominal. The other ten rats were left with 1000 units of hyaluronidase in 5 cc. of distilled water. Only a single medication with hyaluronidase or distilled water was used.

On the eleventh post-operative day the rats were sacrificed and then autopsied. The results are shown in Table X.

X A

SPECIMEN	Multiple Kelly Crush of Caecum + 5 cc. Distilled Water						
	LL	O.toL	O.toB	O.toW	P.O.toB	P.O.toW	Other
All	-	-	-	-	x	-	-
Bll	-	-	-	-	x	-	-
Cll	-	-	-	-	x	-	-
Dll	-	-	-	-	x	-	-
Ell	-	-	x	-	z	-	-
Fll	-	-	x	x	x	-	-
Al2	-	-	x	x	-	-	-
Bl2	-	-	x	-	-	-	-
Cl2	-	-	-	-	x	-	-
Dl2	-	x	-	-	-	-	-

Composite 10 rats 14 Adhesions
Average 1.4 Adhesions

All rats with adhesions.

X B

SPECIMEN	Multiple Kelly Crush of Caecum + 5 cc. D.W. with 100 u. Hyaluronidase.						
	LL	O.toL	O.toB	O.toW	P.O.toB	P.O.toW	Other
E12	-	-	-	-	-	-	-
F12	-	-	-	-	x	-	-
G12	-	-	x	-	-	-	-
H12	-	-	x	-	-	-	-
I12	-	-	x	-	x	-	-
J12	-	-	x	-	x	-	-
K12	-	-	-	-	x	-	-
L12	-	-	x	x	x	-	-
M12	-	-	x	-	x	-	-
N12	died post operative						
	0	0	6	1	6	0	0

9 Rats 13 adhesion Average 1.4
1 Rat - no adhesion

X C

SPECIMEN	Multiple Kelly Caecal Crush + 5 cc. D.W. with 1000 u. Hyaluronidase						
	LL	O.toL	O.toB	O.toW	P.O.toB	P.O.toW	Other
O12	-	-	-	-	x	-	-
P12	-	-	x	-	x	-	-
Q12	-	-	x	x	x	x	-
R12	-	-	-	-	-	-	-
S12	-	x	-	-	x	-	-
T12	-	-	x	-	x	-	-
U12	-	-	x	x	-	-	-
V12	-	-	x	-	x	-	-
W12	d. early pneumonia						
X12	d. early pneumonia						
	0	1	5	2	6	1	

8 rats 15 adhesions average 1.88
1 rat - no adhesion

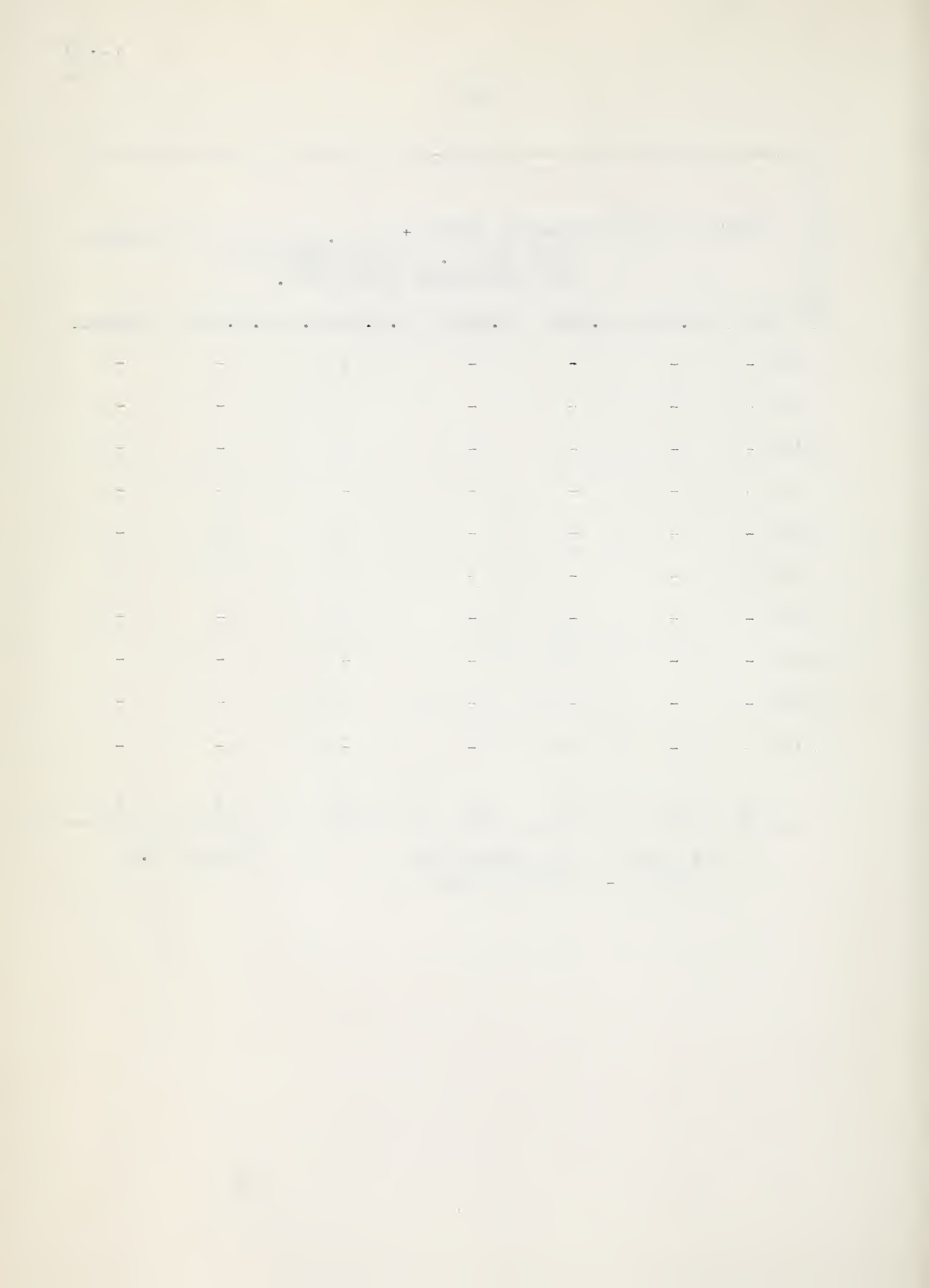
CHAPTER IV

Hyaluronidase, because of its spreading effect may accelerate the activity of such bacteria as the staphylococcus, steptococcus, pneumococcus and the colon bacillus Cl. welchii and the Vibrio septique fatale. The accelerated activity of these organisms may cause increased intraperitoneal damage and further stimulus to adhesion formation. They may be present as contaminants either from outside or from the bowel. Because of this possibility, it was considered advisable to set up a series of ten rats with caecal multiple crush and after giving 100 units of hyaluronidase intraperitoneally in 5 cc. of distilled water to also give intramuscularly, at the time and daily for nine more subsequent days, combiotic. The daily dose per rat was Crystalline penicillin G 7500 units, Crystalline penicillin G Potassium 2500 units; Dihydro streptomycin crystalline sulfate 12.5 mg. These rats were sacrificed on the eleventh day and autopsied;the results are shown in Table XI.

XI A

SPECIMEN	Multiple Kelly Caecal Crush + 5 cc. of Distilled Water with 100 u. of hyaluronidase 10 injections Combiotic.						
	LL	O.toL	O.toB	O.toW	P.O.to B.	P.O.toW	Other
A13	-	-	-	-	x	-	-
B13	-	-	-	-	x	-	-
C13	-	-	-	-	x	-	-
D13	-	-	-	-	-	-	-
E13	-	-	-	-	x	-	-
F13	-	-	-	-	x	-	x
G13	-	-	-	-	x	-	-
H13	-	-	x	-	-	-	-
I13	-	-	-	-	x	-	-
J13	-	-	x	-	-	-	-
	0	0	2	0	7	0	1

10 rats 10 adhesions Average 1.0
1 rat - no adhesions



Because of the action of the adrenal corticoids toward suppressing fibroblastic activity, it was thought to be advisable to try a combination of corticoid and hyaluronidase therapy against adhesion formation. No reports on such trials have been recorded by other investigators.

A series of ten rats was set up in which the caecum was subjected to multiple Kelly crush at laparotomy. These rats on closure were each left with 100 units of hyaluronidase and 5 cc. of distilled water intraperitoneal. Five units of cortisone were given intramuscularly on closure and daily for the nine post-operative days.

On the eleventh day the animals were sacrificed and autopsied. The results at autopsy are shown in Table XI.

SPECIMEN	Multiple Kelly Caecal Crush + 5 cc. D.W. with 100 u. Hyaluronidase, 10 injections Cortisone 5 mgm.						
	LL	O.toL	O.toB	O.toW	P.O.toB	P.O.toW	Other
A15	escaped						
B15	d. 9th d.autopsied		(x)	-	x	-	-
C15	d.11th d.autopsied		(x)	-	x	-	-
D15	-	-	-	-	x	-	-
E15	-	-	-	-	x	-	-
F15	-	-	-	-	x	x	-
G15	-	-	x	-	-	-	x
H15	-	-	-	-	-	-	-
I15	-	-	-	-	x	-	-
J15	-	-	x	x	x	-	-
	0	0	4	1	7	1	1

9 rats 14 adhesions Average 1.6
1 rat - no adhesion

The corticoid influence can be accurately gained by giving ACTH to the animal. The adrenals are stimulated to produce larger amounts of the natural products. This results in the presence in the animal of larger amounts of all the natural corticoids and provides a more balanced influence. Another series of ten rats were subjected to laparotomy and multiple caecal crush. At closure as in the previous two series, 100 units of hyaluronidase in 5 cc. of distilled water was left intraperitoneal in each rat. Two units of ACTH was administered intramuscularly and this was repeated daily until ten doses had been given. On the eleventh day the animals were sacrificed and autopsied. The results of the series are shown on Table XI.

XI C

SPECIMEN	Multiple Kelly Caecal Crush + 5 cc. D.W. with 100 u. Hyaluronidase, 10 injections 2 u. ACTH.						
	LL	O.toL	O.toB	O.toW	P.O.toB	P.O.toW	Other
A14	-	-	x	x	x	x	-
B14	-	-	-	-	x	-	-
C14	-	-	-	-	x	-	-
D14	-	-	-	-	-	-	-
E14	-	-	x	-	x	-	-
F14	-	-	x	-	x	-	x
G14	-	-	x	-	x	-	-
H14	-	-	-	-	-	-	-
I14	-	-	x	-	x	-	x
J14	-	-	-	-	x	-	-
	0	0	5	1	8	1	2

10 rats 17 adhesions Average 1.7
2 rats no - adhesions

CHAPTER V

It is interesting to note that in Table I there appears to be a direct relationship between the amount of talc and the number and density of adhesions. Talc 250 mg. was chosen as the optimum amount because of the relative uniformity of the adhesions formed. Of interest also to note is the little variation in the number of adhesions with prolonged exposure. Apparently the influence of the talc acts immediately to cause adhesion development.

Tables II would indicate that hyaluronidase, if effective is optimum at 100 units. The average number of adhesions was the same for single and repeated doses. The number of adhesions was greater with repeated injections in three groups and less in one group at the various other dosages of hyaluronidase.

In the rats which were used in Table I, several had interlobar adhesions of the liver. This was only infrequently encountered in subsequent series.

The incidence of wound infection and ulceration showed a marked increase in proportion to the increase of hyaluronidase dosage.

All injections were made in the right lower abdominal wall using a No. 21 needle.

Tables III A and B show a considerable difference in the average number of adhesions with single and repeated treatments.

Those with single treatment at laparotomy averaged 0.8 adhesions, those with 100 units at laparotomy and nine subsequent intraperitoneal injections of 100 units each, averaged 1.75 adhesions. However similarly the controls, those with single treatment with 5 cc. distilled water averaged 1.05 adhesions and those receiving 5 cc. initially and nine subsequent injections averaged 1.3 adhesions.

In these two series, a single treatment at the time of laparotomy, with distilled water 5 cc. and 100 units of hyaluronidase, resulted in the lowest average number of adhesions and the greatest number of adhesion-free rats. Even in this series, fourteen out of twenty rats or seventy per cent formed adhesions.

The figures in Table V do not show any particular advantage from the much larger amounts of hyaluronidase used.

Table VI does not reveal any particularly significant findings. It was enlightening to observe however, how little difference resulted from leaving a smooth everted peritoneal line and a raw inverted suture line.

Table VII A seemed to reveal very encouraging results until VII C was charted. The technique had failed to cause adhesion formation. It would have been interesting to observe what would have occurred had a further control group of rats been available with the same surgical treatment but with no fluid or talc left in the abdomen.

In Table VII B, the rats with larger doses of hyaluronidase showed a slightly higher average adhesion formation.

Of the twelve animals used in the series listed in Tables VIII A and B, only fifty per cent formed adhesions, although they were subjected to laparotomy and extensive caecal damage. In several the crush marks were still visible on the caecum at autopsy yet adhesion formation had not developed.

In Table IX A, it is noted that 100 per cent of rats formed one type of adhesion, that of pelvic organ to bowel. In this case the bowel area was the caecum. The method of crushing the caecum is illustrated in Appendix II.

A noticeable difference in adhesion distribution is observed in comparing the distribution in the series of Chapter I and Chapter III.

In Tables X, it will be found that the use of hyaluronidase, 100 units, did not vary the average number of adhesions from that observed in rats receiving distilled water only. Again large doses of hyaluronidase were associated with a higher incidence of adhesion formation.

Comparing the results listed in Tables X A,B.C, and XI A,B, and C, each showing results of surgical crush of the caecum and various treatments, the lowest incidence of

adhesions occurred in the series receiving hyaluronidase and combiotic. One speculates on what would have been found had a series been treated with combiotic alone.

The adhesions observed in the rats of series XI A, were generally much less dense than those observed in the other series. The animals were in excellent health, the peritoneum was of good color and texture and free of oedema.

The poorest animals of all were those receiving hyaluronidase and cortisone. Their coats were rough; the peritoneum was oedematous in most of them.

In both series listed in XI B and XI C Tables, the incidence of adhesions was relatively high.

Variable factors which have been ignored are the influence of pH and of electrolytes. The pH of the peritoneal cavity fluid is difficult to determine under normal conditions and the duration of variation by injected fluids or exudate or transude was beyond the scope of this investigation.

Because chloride ion is an accelerator and sodium ion a depressor of hyaluronidase action, little advantage could be considered from replacing distilled water by saline. Distilled water was considered as causing less pH disturbance and also being hypotonic to possibly hasten absorption.

No attempt has been made to use standard deviation or standard error calculations. Every effort was made to use controls. It was considered so many unseen factors were acting inside the peritoneal cavity that results could not be regarded too objectively. To arrive at any conclusions based on the narrow margins of variations would be erroneous. The only criterion is - Did adhesions form? The answer is "yes", under all circumstances.

Hyaluronidase, at moderate dosage and in single application, in most series showed a lower incidence of adhesion formation. It is however not a solution to the 'adhesion problem'. It may be a step in the proper direction.

The best results were observed when hyaluronidase was used in conjunction with an antibiotic (combiotic-penicillin and streptomycin combination).

The animals in which there was used a combination of hyaluronidase and corticoid factor were in the poorest health, based however on subjective observations.

In Table II F, increasingly larger doses of hyaluronidase would appear to contribute to activity of infection in the healing wound.

To what extent anti-hyaluronidase is a factor is unknown. The inferior results noted when repeated treatments with hyaluronidase were used may be related to the stimulation of anti-hyaluronidase production.

CHAPTER VI

Until further knowledge is gained regarding the relationship of Vitamin C, ground substance, the corticoids, hyaluronidase and possibly related enzymatic compounds, the answer to chemical prevention of adhesions is likely to elude establishment.

For the present, observation of good surgical technique offers the best control. The frequency with which the omentum participated in adhesion formation would suggest that omentum amputation has some merit.

Hyaluronidase, although it may be an aid in reducing adhesion incidence, is not a complete preventative to adhesion formation.

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Appendix I

Technique of laparotomy and talc and fluid instillation.

Female albino Wistar rats were used throughout. Weight ranged from 175 grams to 250 grams. The rats were permitted to feed as desired on mash made of Fox Checkers (see Appendix IV); water was available ad. lib.

The day preceding laparotomy each rat was clipped of hair on the abdomen. The day of laparotomy, food and fluid was withheld until after laparotomy. All materials used were sterilized. Aseptic technique was used. The abdomens were prepared with either one per cent iodine or tincture of merthiolate.

All animals received preliminary anaesthesia in an ether jar and then an assistant fixed the animal by elastic bands from the legs to the four corner posts of the rat board. Anaesthesia was continued by placing a crucible lined with ether soaked four gauze over the animals head as necessary.

A sterile drap with operating aperture was placed over the rat abdomen after the assistant had applied iodine or merthiolate, to the area.

The abdomen was opened by a one inch longitudinal mid-line incision in the mid region of the abdomen. This incision

was deepened to open the recti muscles and the peritoneum.

The margin of the incision was then retracted and elevated, the delivery tip of the funnel inserted and the previously prepared sterilized portion of talc dusted from the testtube, into the funnel by the assistant. The funnel was removed and then the assistant injected the proper amount of distilled water or solution of hyaluronidase from a syringe and No. 18 needle into the abdomen through the partially closed incision.

Closure was obtained by using continuous black silk or cotton. The suture was carried through the skin and then used to close the peritoneum and muscle layers in a continuous running everting suture. This suture was then carried through the skin to the exterior and carried back along the skin as a continuous suture to the point of original skin puncture. Here it was tied to the trailing end of the suture.

The incision was left uncovered. The animals were placed in a recovery cage and identification was maintained, either by ear clips or marked cages.

Appendix III

Technique of sacrifice and autopsy:

The rats were placed in ether bell jars for at least one-half hour. When evidently dead, the rats were then placed in a refrigerator at thirty-two degrees Fahrenheit.

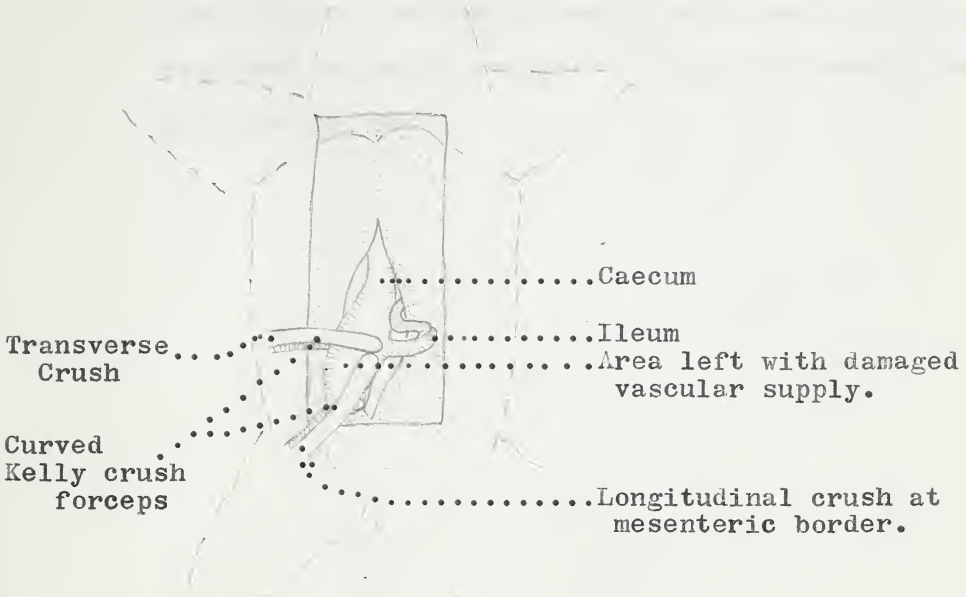
When all of the group was prepared the rats were removed from the refrigerator and after two to four hours were autopsied.

A Transverse incision was made from one axillary line to the other at the level of the costal margin. The serosal surface of the abdominal wall was then inspected and opened longitudinally so as to avoid disturbing the suture line and any adhesion.

The four flaps were then widely retracted and a thorough inspection made. Particular types of adhesions were noted -

Appendix II

Caecal Crush



The laparotomy was performed as previously described in Appendix I. However, the incision was longer and located somewhat more caudad. Closure was in the same fashion as described in Appendix I.

omentum to liver and stomach to liver; occlusion of the liver lobes; omentum to bowel, and bowel and/or omentum to abdominal wall; and pelvic organs to bowel, omentum or abdominal wall.

Appendix IV

Fox Checkers, ordered from Ralston ~~and~~ Purina, Woodstock, Ontario.

Ingredients:

Vitamin B12 Supplement, Riboflavin Supplement, Thiamin, D Activated Plant Sterol, Vitamin A Feeding Oil, Wheat Germ, Dried Skimmed Milk, Animal Liver Meal, Brewers' Dried Yeast, Fish Meal, Meat Meal, Dehydrated Alfalfa Meal, Cooked Wheat Grits, Soybean Oil Meal, Molasses, Dried Beet Pulp, Manganese Sulphate, Curacao Rock Phosphate, 5 % iodized Salt.

Guaranteed Analysis:

Crude Protein, not less than	20.0%
Crude fat, not less than	3.0%
Crude fibre, not more than	6.0%

Appendix V

Materials

Hyaluronidase - Diffusin, Ortho brand hyaluronidase, was used throughout the investigation. The preparation is measured quantitatively in units - Turbidity Reducing Units, written as T.R.U. One turbidity reducing unit is equal to 3.33 Viscosity Reducing units. In this presentation where unit is used it refers to Turbidity Reducing Unit.

Distilled Water - The distilled water used in the investigation was obtained from the laboratory reservoirs and was distilled in the laboratory.

Talc - The talc was obtained in bulk from the pharmacy of the University of Alberta Hospital, Edmonton. It is the same preparation as that which was formerly used in surgeon's gloves.

ADHESIONS FROM TALC

Pelvic Organ to Bowel

Pelvic Organ to Bowel to Abdominal Wall

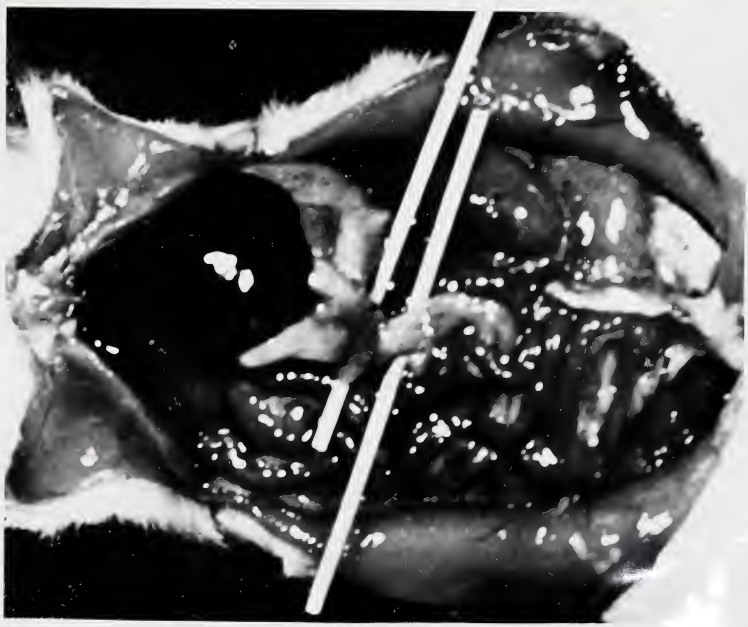
ADHESIONS FROM TALC

Omentum to Liver

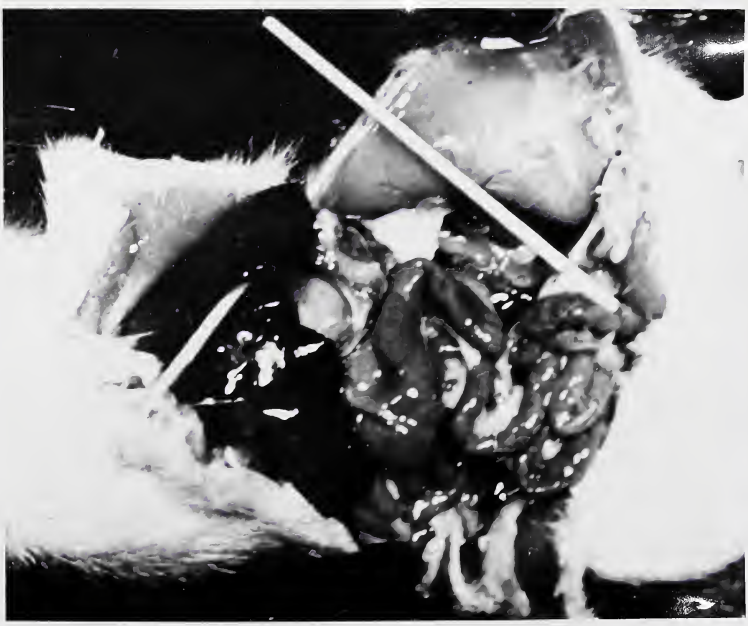
el

Liver lobes, Liver to Omentum, to Bowel

ADHESIONS FROM TALC

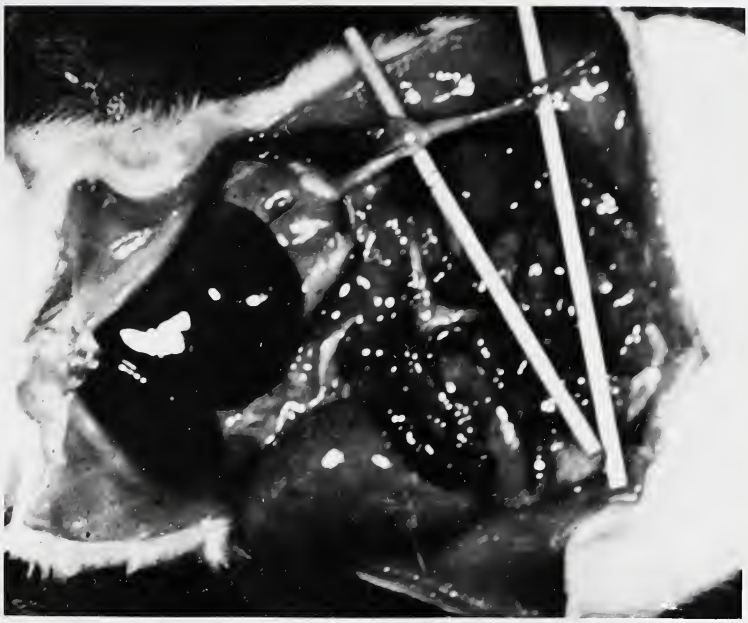


Omentum to Bowel and Abdominal Wall

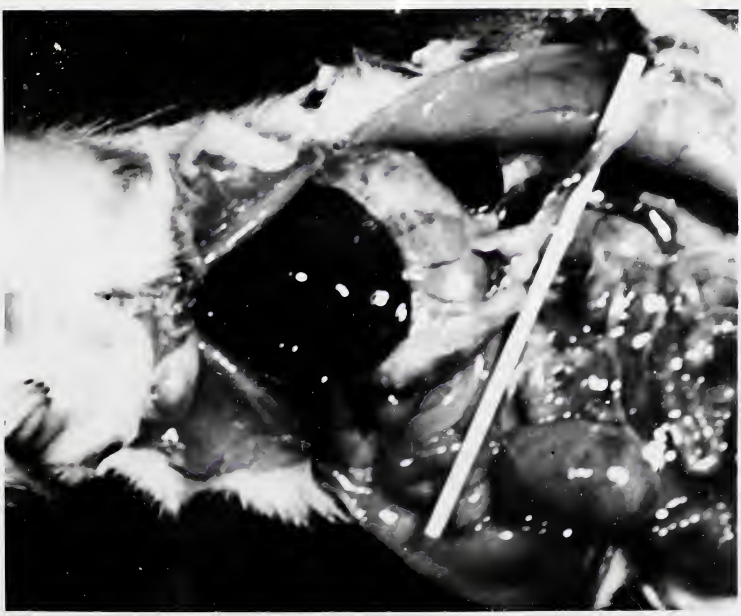


Omentum to Liver, Stomach and Mesentery

ADHESIONS FROM TALC



Omentum to Abdominal Wall



Omentum to Stomach, Abdominal Wall

Submitted by,

Alvin W. Mooney, B.A., M.D.

B29771